REMARKS

Claims 1-5 are pending in the instant application. Claims 3-5 have been withdrawn from consideration and canceled. Claims 1-2 have been rejected. Claim 1 has been amended. No new matter has been added by this amendment. Reconsideration is respectfully requested in light of the following remarks.

I. Election/Restriction Requirement Under 35 U.S.C. §121

The Examiner suggests that the inventions of Group I and Group II do not share the same or corresponding technical feature of a 34 kDa truncated plasmin proteolytic protein because this technical feature is not special since it was known in the prior art and therefore cannot make a contribution over the prior art. Thus, the restriction requirement placing the claims into Groups I and II has been deemed proper and made final. Claims 3-5 are withdrawn from further consideration. To facilitate the prosecution of this application, Applicant is canceling claims 3-5 without prejudice, reserving the right to file continuing applications for the canceled subject matter.

II. Rejection of Claims Under 35 U.S.C. §112

Claims 1-2 are rejected under 35 U.S.C. 112, first paragraph, because it is suggested that while the specification is enabling only for a method for producing a 35 kDa truncated plasmin proteolytic protein comprising combining plasminogen and porcine or human plasminogen activator inhibitor 1 lacking a partial heparin-binding domain and RCL domain (rPAI-1_{23}) for two hours at 37°C and subsequently adding urokinase plasminogen activator (uPA) for an additional hour at 37°C so that a 34 kDa

truncated plasmin proteolytic protein (angiostatin) is produced and a 34 kDa truncated plasmin proteolytic protein produced by said method, the specification does not provide enablement for the method as recited in claim 1 and any 34 kDa truncated plasmin proteolytic protein as recited in claim 2 for treating angiogenesis. It is suggested that the specification does not enable any person skilled in the art to which it pertains to make and use the invention commensurate in scope with the claims. In particular, the Examiner suggests that the specification only discloses one method for producing a 34 kDa truncated plasmin proteolytic protein and the essential material rPAI-1_{23} has been improperly incorporated by reference. It is suggested that Applicant amend the disclosure to include the material incorporated by reference, wherein the amendment is accompanied by an affidavit or declaration executed by Applicant or practitioner representing Applicant, state that the amendatory material consists of the same material incorporated by reference in the referencing application. The Examiner also suggests that other than the specific conditions mentioned above to produce the claimed 34 kDa truncated plasmin proteolytic protein, there is insufficient guidance as to the concentration of reagents, the duration of incubation and the temperature at which the reaction takes place to consistently produce the claimed protein. The Examiner further suggests that without amino acid sequence, there is insufficient guidance as to the structure of the truncated plasmin proteolytic protein. It is also suggested there is insufficient guidance and *in vivo* working example demonstrating that the 34 kDa truncated plasmin proteolytic protein has anti-angiogenic effect *in vivo* for a method of treating diseases

related to angiogenesis because *in vitro* protocols are not predictive of treatment *in vivo* malignancies, as evidenced by the teachings of Stryer et al., Dermer et al., and Gura et al.

Claims 1-2 have also been rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. Specifically, it is suggested that the specification does not reasonably provide a written description of the structure of any and all 34 kDa truncated plasmin proteolytic protein produced by the claimed method, does not provide written description for the structure of rPAI-1_{23} and uPA, and does not provide written description for the conditions such as time, temperature and concentration of each reactant in the claimed method. Applicant respectfully traverses these rejections under 35 U.S.C. 112, first paragraph.

Applicant has appreciated that the order in which uPA and rPAI-1_{23} are added to plasminogen has a substantial effect on the plasmin products produced by the reaction. In particular, Applicant has found that uPA can compete rPAI-1_{23} away from plasminogen, thereby diminishing plasminogen fragments containing K1-3 at 34 kDa (*i.e.*, the 34 kDa truncated plasmin proteolytic protein). See page 19, lines 20-26. Accordingly, Applicant has used this insight to provide parameters for consistently producing a 34 kDa truncated plasmin proteolytic protein from plasminogen. In this regard, page 29 (lines 5-26) teaches that conversion of plasminogen to plasmin by rPAI-1_{23} with subsequent conversion of plasmin to 34 kDa truncated plasmin proteolytic

protein by the addition of uPA, in particular single chain uPA (see page 26, lines 5-8). This passage further discloses that the amount of 34 kDa truncated plasmin proteolytic protein can be varied by varying parameters such as reaction time and amount of reactants. For example, "Longer incubation times may be necessary when lower concentrations of rPAI-1_{23} are used to enhance the conversion of plasminogen to plasmin." See page 39, lines 16-26. Moreover, "intensity of proteolytic activity near 34 kDa was much greater when uPA was reacted with the rPAI-1_{23} and the plasminogen reaction mixture for 45 minutes as compared to shorter times with uPA in the mixture", *e.g.*, 15 and 30 minutes. See page 28, lines 8-11. Thus, the specification provides the necessary guidance to the skilled artisan as to how to make and use the invention, wherein parameters such as the "specified amount of time", temperature, and amount of reactants are selected by the skilled artisan depending on the desired amount of 34 kDa truncated plasmin proteolytic protein to be produced.

Regarding the nature of the essential material rPAI-1_{23}, Applicant submits herewith a declaration by Dr. Mary Jo Mulligan-Kehoe which indicates that the DNA encoding the truncated PAI-1_{23} protein of the invention was obtained by PCR amplification of a fragment of the porcine PAI-1 gene (*poPAI-1*) based on the *poPAI-1* sequences that correspond to the human PAI-1 gene (*huPAI-1*). Amplification of the truncated porcine PAI-1_{23} protein was carried out using the primers described in Mulligan-Kehoe et al. (2001) *J. Biol. Chem.* 276:8588-8596. As presented in paragraph one, column 2, of page 8589 of this reference, the primers were 5'-primer, 5'-GGAATTCAAGGAGCTATGG-3' and 3'-primer, 5'-GCTCTAGATTTCCACTGGTGATG-3'. As disclosed in the fourth full

paragraph of column 1 of page 8589, the amplified fragment encoding the PAI-1_{23} protein corresponds to nucleotides 444-999 of *poPAI-1* and 238-793 of *huPAI-1*. Thus, in an earnest effort to clarify the structure of the PAI-1_{23} protein of the present invention, Applicant has amended Example 1 and submits herewith a substitute Sequence Listing to include the subject matter incorporated by reference in the instant application as originally filed. Support for the incorporation of this subject matter can also be found in the specification as originally filed. For example, Figure 1 and page 11 (lines 4-9) teach that rPAI-1_{Hep23} and rPAI-$1_{\Delta 23}$ share a common C-terminus with rPAI-1_{23}, whereas rPAI-1_{24} shares a common N-terminus with rPAI-1_{23}. In this regard, Example 1 discloses the DNA encoding the rPAI-1_{23} protein by virtue of disclosing the DNA sequence encoding rPAI-1_{Hep23}, rPAI-$1_{\Delta 23}$, and rPAI-1_{24}. See summary table of teachings of Example 1.

Protein	5′ and 3′ primers, respectively	Position in *poPAI-1*	Position in *huPAI-1*
rPAI-1_{Hep23}	GGAATTCATGCAGTTCAAGATTGAGGAGAAGGGC GCTCTAGATTTCCACTGGCTGATG	390-*999*	184-*793*
rPAI-$1_{\Delta 23}$	GGAATTCATGGATGAGATCAGCACGG GCTCTAGATTTCCACTGGCTGATG	471-*999*	265-*793*
rPAI-1_{24}	GGAATTCAAGGAGCTCATGG GCTCTAGATCAAGGCTCCATCAC	*444*-1346	*238*-1162

Thus, it is clear from these teachings, that the DNA encoding the PAI-1_{23} protein corresponds to nucleotides 444-999 of *poPAI-1* and 238-793 of *huPAI-1*. To further clarify the structure of the nucleotide sequences encoding rPAI-1_{23}, Applicant has amended the claims and incorporated into the Sequence Listing (SEQ ID NO:3 and SEQ ID NO:4) the respective porcine and human cDNA sequences of Accession Nos. Y11347 and

J03764, as disclosed in the specification at page 60, lines 26 and 29.

As to the structure of uPA (*i.e.*, urokinase plasminogen activator), this protein was well-known and commercially available at the time of filing of the instant invention as evidenced by the teachings of Planus et al. (1997) *J. Cell Sci.* 110:1091; enclosed herewith). Planus et al. teach in the legend of Figure 3 that soluble uPA was commercially available from American Diagnostica. Accordingly, uPA was readily available to the skilled artisan at the time of filing of the instant application.

As to the structure of the 34 kDa truncated plasmin proteolytic protein, Applicant respectfully disagrees with the Examiner. Compliance with written description requirement of 35 U.S.C. 112, first paragraph, may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention. For some biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length. Although structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. See MPEP §2163. At the time of filing of the instant application, the structure of plasmin was well-known and purified plasmin was commercially available (see page 29, lines 3-4). In this regard, the specification clearly discloses that the claimed protein is a truncated version of plasmin, which is 34 kDa, exhibits

proteolytic activity (see page 29, lines 10-13) and is recognized by a mini-plasminogen antibody (see page 17, lines 18-20). Accordingly, given this combination of identifying characteristics, one of skill in the art would readily recognize that Applicant was in possession of the disclosed protein. In an earnest effort to further clarify the nature of the 34 kDa truncated plasmin proteolytic protein, Applicant has amended claim 1, as supported by the disclosure at page 17 (lines 18-20), to indicate that this protein is recognized by a mini-plasminogen antibody. The specification teaches at page 64 (lines 30-34) that a mini-plasminogen antibody, *e.g.,* provided by American Diagnostica, Greenwich, CT) is defined as an antibody that binds to kringle 5 plus the serine protease domain and not to complexed plasmin.

Cleavage products of plasminogen, *e.g.,* angiostatin, were well-known in the art for their anti-angiogenic activity and use *in vivo* for the treatment of cancer. For example, Soff ((2000) *Cancer Metastasis Rev.* 19(1-2):97-107; abstract submitted herewith) indicates that recombinant angiostatin K1-3 (kringles 1-3) was being used in phase 1 clinical trials for cancer treatment, as well as phase 1 trials of an Angiostatin Cocktail, which induces the direct *in vivo* conversion of plasminogen to angiostatin 4.5 (kringles 1-4, plus most of kringle 5). MPEP 2164.03 indicates that the amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability in the art. In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The "amount of guidance or direction" refers to that information in the application, as originally filed, that teaches

exactly how to make or use the invention. The more that is known in the prior art about the nature of the invention, how to make, and how to use the invention, and the more predictable the art is, the less information needs to be explicitly stated in the specification. In this regard, as a cleavage product of plasminogen, which exhibits *in vitro* anti-angiogenic activity like angiostatin, the instant 34 kDa truncated plasmin proteolytic protein would predictably have an *in vivo* anti-angiogenic effect.

Because Applicant has provided a clear description of how to make and use the instant invention and further conveyed with reasonable clarity to those skilled in the art that, as of the filing date sought, Applicant was in possession of the instant invention, Applicant has met both the enablement and written description requirements. It is therefore respectfully requested that these rejections be reconsidered and withdrawn.

Claims 1-2 have further been rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. In particular, it is suggested that "rPAI-1_{23}" and "uPA" are ambiguous and indefinite because different laboratories may use the same laboratory designations to define completely distinct polypeptides. Applicant respectfully disagrees with this rejection.

As discussed above, the PAI-1_{23} protein corresponds to nucleotides 444-999 of *poPAI-1* (SEQ ID NO:3) and 238-793 of *huPAI*-1 (SEQ ID NO:4). The claims have been amended to clarify this structure and further amended to indicate that "rPAI-1" stands for "recombinant plasminogen activator inhibitor-1".

Support for this amendment is found at page 3 (line 31) and page 10 (line 17). Moreover, to clarify what is meant by "uPA", Applicant has amended claim 1 to read on a "urokinase plasminogen activator" as supported by the specification at page 3 (line 12-13) and page 26 (lines 5-8). It is therefore respectfully requested that this rejection be withdrawn.

Claims 1-2 have been rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such as omitting the specified amount of time before adding uPA, the temperature at which the reaction takes place, and the concentration of each reactant. Applicant respectfully traverses this rejection. As discussed above, the essential step of the instant method is the order in which the reactants are added to the reaction. The specified amount of time, temperature and concentration of reactants are variable and dependent upon the amount of 34 kDa truncated plasmin proteolytic protein desired by the skilled artisan. It is therefore respectfully requested that this rejection be reconsidered and withdrawn.

III. Rejection of Claims Under 35 U.S.C. §102

Claims 1-2 have been rejected under 35 U.S.C. 102(b) as being anticipated by Mulligan-Kehoe et al. (2001) *J. Biol. Chem.* 276(11):8588-8596 (PTO 892). The Examiner suggests that Mulligan-Kehoe et al. teach a 34 kDa truncated plasmin proteolytic protein produced by cleavage of plasmin by rPAI-1_{23} regardless of the permutation for combining rPAI-1_{23}, uPA and plasmin. It is suggested that Mulligan-Kehoe et al. disclose a method of making truncated plasmin proteolytic protein comprising combining plasminogen and rPAI-1_{23} for 1 hour at 37°C, then adding uPA for

an additional 1 hour at 37°C (page 8590, col. 2, first paragraph). The Examiner suggests that given that the claimed method steps are the same as the reference method steps, the reference method inherently produces the same 34 kDa truncated plasmin proteolytic protein. Applicant respectfully disagrees with this rejection.

MPEP 2131 states that "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

While Mulligan-Kehoe et al. disclose a reaction containing plasminogen and PAI-1_{23} with subsequent addition of uPA, this reference does not teach or suggest the nature of the uPA molecule. In contrast, Applicant has appreciated that a single chain uPA provides a 34 kDa truncated plasmin proteolytic protein, whereas two-chain uPA produces a slight upward shift in the molecular weight of the protein (see page 26, lines 5-12). Given the lack of such a side-by-side comparison in Mulligan-Kehoe et al., it is unclear whether the protein of Mulligan-Kehoe et al. represents the 34 kDa truncated plasmin proteolytic protein of the instant invention, which is recognized by a mini-plasminogen antibody, or the slightly larger protein disclosed in the specification. Accordingly, in an earnest effort to clarify the nature of the uPA protein employed in the instant method for producing the claimed 34 kDa truncated plasmin proteolytic protein, Applicant has amended claim 1 to indicate that the uPA protein is a single chain uPA as supported by the disclosure at page 26 (lines 5-12). Because Mulligan-Kehoe et al. fail to teach

or suggest a single chain uPA for use in producing a 34 kDa truncated plasmin proteolytic protein which is recognized by a mini-plasminogen antibody, this reference cannot be held to anticipate the instant invention. It is therefore respectfully requested that this rejection be withdrawn.

Claim 2 has been rejected under 35 U.S.C. 102(b) as being anticipated by U.S. Patent No. 5,801,146. The Examiner suggests that the '146 patent teaches a truncated plasmin proteolytic protein such as angiostatin containing kringles 1-3 (col. 2, lines 17-19). It is suggested that the reference protein inherently has the same 34 kDa as evidenced by the specification at page 14, lines 20-22. Applicant respectfully traverses this rejection.

The '146 patent discloses at col. 2, lines 17-19, a polypeptide containing kringles 1-3. This reference does not teach or suggest how the protein is produced nor does this reference teach or suggest the molecular weight, activity, or antibody specificity of this protein. In contrast, the instant specification discloses a 34 kDa truncated plasmin protein which exhibits proteolytic activity and is recognized by a mini-plasminogen protein. Because this reference does not teach or suggest each and every element of the claimed protein, this reference cannot be held to anticipate the instant invention. It

is therefore respectfully requested that this rejection be withdrawn.

Respectfully submitted,

Jane Massey Licata
Registration No. 32,257

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Licata & Tyrrell P.C.
66 E. Main Street
Marlton, New Jersey 08053

(856) 810-1515